Despite these limitations, this study demonstrates the high potential for HEV to cause outbreaks in communities with recently displaced persons. Of note, all of the reported outbreaks in this study occurred in the context of highly crowded camps or settlements, supporting the association between hepatitis E outbreaks and those environments. Given that some of the outbreaks noted in this analysis appeared to cross national borders, genetic sequencing to validate related strains may be useful for disease surveillance and prevention efforts. Additional data are needed to evaluate the potential utility of HEV vaccination in outbreaks and the barriers to vaccinating residents of refugee and IDP settlements. Water, sanitation, and hygiene measures are critical to reducing disease outbreaks, as is improved cross-border communication to prevent and manage future outbreaks. Clinicians and relief staff working with displaced populations should be vigilant for signs of hepatitis E disease, particularly among high-risk hosts such as pregnant women. Resources must be devoted to improving HEV surveillance, diagnostic capabilities, and response efforts for refugee and displaced populations.

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About the Author

Dr. Desai is an infectious disease physician and researcher whose primary focus is the application of informal surveillance methods for displaced populations, as well as for emerging and reemerging infectious disease threats.

References

1. Bagulo H, Majekodunmi AO, Welburn SC. Hepatitis E in sub-Saharan Africa—a significant emerging disease. One Health. 2020;11:100186. https://doi.org/10.1016/j.onehlt.2020.100186
2. UN High Commission for Refugees. Global trends: forced displacement in 2017. 2017 [cited 2019 May 30]. http://www.unhcr.org/en-us/statistics
3. Browne LB, Menkir Z, Kahi V, Maina G, Asnakew S, Tubman M, et al.; Centers for Disease Control and Prevention (CDC). Notes from the field: hepatitis E outbreak among refugees from South Sudan—Gambella, Ethiopia, April 2014-January 2015. MMWR Morb Mortal Wkly Rep. 2015;64:537.
4. Lagare A, Ibrahim A, Ousmane S, Issaka B, Zaneidou M, Kadadé G, et al. Outbreak of hepatitis E virus infection in displaced persons camps in Diffa Region, Niger, 2017. Am J Trop Med Hyg. 2018;99:1055–7. https://doi.org/10.4269/ajtmh.17-0950
5. Bijkerk P, Monnier AA, Fanoy EB, Kardamanidis K, Friesema IH, Knol MJ. ECDC round table report and ProMed-mail most useful international information sources for the Netherlands Early Warning Committee. Euro Surveill. 2017;22:pii=30502. https://doi.org/10.2807/1560-7917.ES.2017.22.14.30502
6. World Bank Group. Sub-Saharan Africa [cited 2019 May 28]. http://data.worldbank.org/region/sub-saharan-africa

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Usutu Virus Africa 3 Lineage, Luxembourg, 2020

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We detected Usutu virus in a dead Eurasian blackbird (*Turdus merula*) in Luxembourg in September 2020. The strain clustered within the Africa 3.1 lineage identified in Western Europe since 2016. Our results suggest maintenance of the virus in Europe despite little reporting during 2019–2020, rather than a new introduction.

West Nile virus (WNV) and Usutu virus (USUV), members of the family *Flaviviridae*, share several epidemiologic traits and cocirculate in Europe. Both viruses are maintained through a transmission cycle involving bird and mosquito vectors. Migratory birds likely play a role in long-distance spread of USUV, similarly to WNV, and in the recent introduction of the virus to Europe from Africa (*1*).

In Europe, USUV has been associated with bird dieoff events since 2001 (*2*) and seems notably pathogenic for passerines and owls (*3*). Massive dieoff

events of Eurasian blackbirds (*Turdus merula*) have become a hallmark of USUV circulation in Western Europe, enabling its detection through passive surveillance (*2*,*4*,*5*).

WNV and USUV are also occasionally transmitted through a mosquito bite to mammals (such as humans or horses), which are considered dead-end hosts (*3*) and experience a wide range of clinical signs up to neuroinvasive syndromes. Although most persons infected with USUV experience no or limited symptoms, USUV can cause more severe disease in certain persons or be detected in blood donations with yet-unknown consequences for the blood product recipients (*6*). The apparent intense virus circulation in countries neighboring Luxembourg that began in 2016, coupled with accumulating reports of USUV infections in humans (*7*), prompted us to initiate passive surveillance in Luxembourg as an early warning system for mosquitoborne *Flaviviridae* circulation.

During October 2018–September 2020, a total of 61 samples from 33 birds (Table) were submitted for investigation of WNV or USUV infection. The animals were found dead or died shortly after arrival at a wildlife rehabilitation center. All samples were screened for the presence of WNV and USUV by real-time reverse transcription PCR (Appendix, https://wwwnc.cdc.gov/EID/article/28/5/21-2012-App1.pdf). All tested negative for WNV. In September 2020, one brain sample from a Eurasian blackbird found dead in a home garden near the capital city tested positive for USUV (cycle threshold 22.09) (Table). Before death, the animal exhibited neurologic symptoms (disorientation, loss of coordination). The presence of USUV RNA was confirmed by a second real-time reverse transcription PCR test, and the whole genome was sequenced for further strain characterization.

Phylogenetic analyses assigned the USUV strain from Luxembourg to the Africa 3 lineage. This lineage was first identified in Germany in 2014 (*4*); since then, it has been regularly described in Belgium, France, Germany, and the Netherlands (*4*,*5*) and has occasionally been reported in the Czech Republic (2018) (*8*) and the United Kingdom (2020) (*9*) (Figure). More precisely, the USUV strain from Luxembourg grouped within the Africa 3.1 sublineage, which is the least represented lineage (*5*). It clustered together with strains from blackbirds and a common scoter (*Melanitta nigra*) detected in Belgium, Germany, France, and the Netherlands in 2016 and 2018 (Appendix Figure). The intermingling of the only 2 strains reported in 2020 from Luxembourg and the United Kingdom within Africa 3.1 and 3.2 together with earlier Western Europe strains suggests local virus spread rather than a new virus introduction in Europe. However, little reporting in 2019 and 2020 and the lack of sequences from Africa hamper definite conclusion. The time gaps between the estimated ancestors of the Africa 3 lineage (2009) and Europe 3 lineage (2002) (*5*) and the earliest sequences available (2014 for Africa 3 and 2010 for Europe 3) further suggest that silent USUV circulation is not uncommon. In addition, passive surveillance in Luxembourg might have missed earlier cases, as was reposted in Austria, where only an estimated 0.2% of blackbirds killed by USUV were identified during 2003–2005 (*10*).

The transmission of both WNV and USUV is governed by a combination of factors, such as temperature, which influences both the developmental cycles of mosquitoes and virus transmissibility (*10*). Unusually high temperatures likely promoted the unprecedented USUV circulation in Western Europe (*4*,*10*). Expanding USUV geographic distribution is considered by some to be an indicator of WNV dispersion potential (*11*,*12*).

Table. Samples collected in the framework of WNV and USUV passive surveillance, Luxembourg, 2018–2020*

Year	Bird species	Location	No. samples tested	Sample types	No. birds positive/no. total	
					WNV	USUV
2018	*Turdus merula*	Rehabilitation center	4	Liver, brain, kidney, heart	0/1	0/1
	Tyto alba	Rehabilitation center	6	Liver, brain, kidney, heart, tracheal swab, cloacal swab	0/1	0/1
	Pica pica	Esch-sur-Alzette	4	Liver, brain, kidney, heart	0/1	0/1
2019	*T. merula*	Rehabilitation center	10	Brain	0/10	0/10
	Corvus corone	Rehabilitation center	2	Brain	0/2	0/2
	Corvus frugilegus	Rehabilitation center	3	Brain	0/3	0/3
	Corvus sp.	Rehabilitation center	1	Brain	0/1	0/1
2020	*Sturnus vulgaris*	Lamadelaine, Pétange	20	Brain, tracheal swab, cloacal swab	0/9	0/9
	Corvus sp.	Pétange	10	Brain, tracheal swab, cloacal swab	0/4	0/4
	T. merula	Strassen	1	Brain	0/1	1/1
Total			61		0/33	1/33

*USUV, Usutu virus; WNV, West Nile virus.

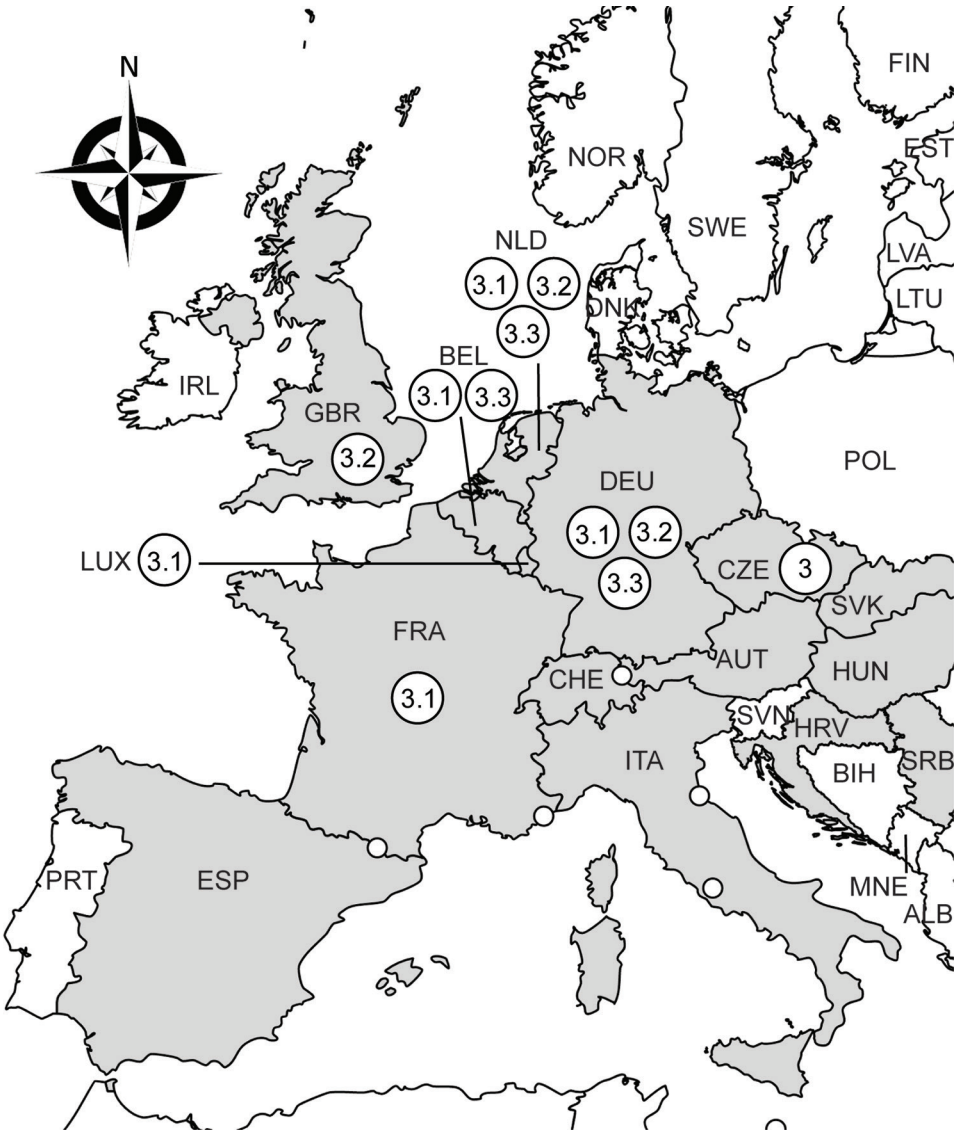

Figure. Geographic distribution of Usutu virus Africa 3 lineage in Europe. Countries are identified by 3-letter International Organization for Standardization codes (https://www.iso.org); gray indicates those where Usutu sequences were reported (partial E gene, partial NS5 gene, or complete polyprotein coding viral sequences available on GenBank). Large white circles indicate locations where Africa 3 lineage has been identified; sublineages are indicated within circles. Only partial NS5 sequence was available for Africa 3 strain from Czech Republic, preventing sub-lineage attribution. Small white circles delineate European microstates (AND, MCO, LIE, SMR and Vatican city); no Usutu virus circulation was reported. Map created with https://www.mapchart.net.

The spread of WNV to Germany in 2018 and the Netherlands in 2020 corroborates this hypothesis. Because of the increasing frequencies of climatic anomalies, Luxembourg is also at risk for WNV to be introduced. Surveillance of mosquitoborne viruses such as USUV and WNV in animal hosts should be maintained and strengthened in the country as an early warning system to inform public health authorities.

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References

1. Engel D, Jöst H, Wink M, Börstler J, Bosch S, Garigliany MM, et al. Reconstruction of the evolutionary history and dispersal of Usutu virus, a neglected emerging arbovirus in Europe and Africa. MBio. 2016;7:e01938–15. https://doi.org/10.1128/mBio.01938-15
2. Weissenböck H, Kolodziejek J, Url A, Lussy H, Rebel-Bauder B, Nowotny N. Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe. Emerg Infect Dis. 2002;8:652–6. https://doi.org/10.3201/eid0807.020094

3. Benzarti E, Linden A, Desmecht D, Garigliany M. Mosquito-borne epornitic flaviviruses: an update and review. J Gen Virol. 2019;100:119–32. https://doi.org/10.1099/jgv.0.001203
4. Cadar D, Lühken R, van der Jeugd H, Garigliany M, Ziegler U, Keller M, et al. Widespread activity of multiple lineages of Usutu virus, western Europe, 2016. Euro Surveill. 2017;22:30452. https://doi.org/10.2807/1560-7917.ES.2017.22.4.30452
5. Oude Munnink BB, Münger E, Nieuwenhuijse DF, Kohl R, van der Linden A, Schapendonk CME, et al. Genomic monitoring to understand the emergence and spread of Usutu virus in the Netherlands, 2016–2018. Sci Rep. 2020;10:2798. https://doi.org/10.1038/s41598-020-59692-y
6. Aberle SW, Kolodziejek J, Jungbauer C, Stiasny K, Aberle JH, Zoufaly A, et al. Increase in human West Nile and Usutu virus infections, Austria, 2018. Euro Surveill. 2018;23:23. https://doi.org/10.2807/1560-7917.ES.2018.23.43.1800545
7. Vilibic-Cavlek T, Petrovic T, Savic V, Barbic L, Tabain I, Stevanovic V, et al. Epidemiology of Usutu virus: the European scenario. Pathogens. 2020;9:E699. https://doi.org/10.3390/pathogens9090699
8. Hönig V, Palus M, Kaspar T, Zemanova M, Majerova K, Hofmannova L, et al. Multiple lineages of Usutu virus (Flaviviridae, Flavivirus) in blackbirds (Turdus merula) and mosquitoes (Culex pipiens, Cx. modestus) in the Czech Republic (2016–2019). Microorganisms. 2019;7:568. https://doi.org/10.3390/microorganisms7110568
9. Folly AJ, Lawson B, Lean FZ, McCracken F, Spiro S, John SK, et al. Detection of Usutu virus infection in wild birds in the United Kingdom, 2020. Euro Surveill. 2020;25:2001732. https://doi.org/10.2807/1560-7917.ES.2020.25.41.2001732
10. Rubel F, Brugger K, Hantel M, Chvala-Mannsberger S, Bakonyi T, Weissenböck H, et al. Explaining Usutu virus dynamics in Austria: model development and calibration. Prev Vet Med. 2008;85:166–86. https://doi.org/10.1016/j.prevetmed.2008.01.006
11. Zannoli S, Sambri V. West Nile virus and Usutu virus co-circulation in Europe: epidemiology and implications. Microorganisms. 2019;7:E184. https://doi.org/10.3390/microorganisms7070184
12. Fros JJ, Miesen P, Vogels CB, Gaibani P, Sambri V, Martina BE, et al. Comparative Usutu and West Nile virus transmission potential by local Culex pipiens mosquitoes in northwestern Europe. One Health. 2015;1:31–6. https://doi.org/10.1016/j.onehlt.2015.08.002

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COMMENT LETTERS

Guillain-Barré Syndrome Associated with COVID-19 Vaccination

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To the Editor: With interest we read the article by Shao et al. (*1*) about the frequency of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination–associated Guillain-Barré syndrome (SCoVaG) among 18,269 healthcare workers in Taiwan who had received the AstraZeneca vaccine (AZV; https://www.astrazeneca.com). Only 1 vaccinee experienced SCoVaG during the study period (*1*). The study is appealing but raises concerns.

Recently, our review of 19 SCoVaG patients, for whom data were collected through June 2021, was published (*2*). The 9 men and 10 women in the study were 20–86 years of age. All patients experienced SCoVaG after the first vaccine dose. AZV was given to 14 patients, the Pfizer-BioNTech (https://www.pfizer.com) vaccine to 4 patients, and the Johnson & Johnson (https://www.jnj.com) vaccine to 1 patient. Latency between vaccination and SCoVaG onset ranged from 3 hours to 39 days. Patients received intravenous immune globulin (n = 13), steroids (n = 3), or no therapy (n = 3). Six patients required mechanical ventilation. One patient recovered completely; 9 achieved partial recovery (*2*). Only 1 of the studies included in our review mentioned the total number of vaccinated persons (*3*); in that study, 7 persons among 1.2 million vaccinated persons were found to have SCoVaG (*3*).

In addition, data on 389 patients with SCoVaG were collected in a recent review about the neurologic adverse events of SARS-CoV-2 vaccination (*4*). However, no individual data were provided for 337

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Usutu Virus Africa 3 Lineage, Luxembourg, 2020

Appendix

Material and Methods

WNV and USUV Detection

Upon sample reception, nucleic acids were extracted from 140 µL of virus transport medium in which cloacal and tracheal swabs were stored immediately after sample collection using the QIAmp viral RNA mini kit (Qiagen, Venlo, the Netherlands). Tissues (30 mg; brain, liver, kidney, heart) (Table, https://wwwnc.cdc.gov/EID/article/28/5/21-2012-T1.htm) were lysed in 600 µL of RLT lysis buffer supplemented with 14.3 M β-mercaptoethanol in the TissueLyser II (Qiagen) with 5 mm steal beads for 2x 30 sec at 30 Hz. After centrifugation for 3 min at 16 000 g to remove tissue debris, nucleic acids were purified from the supernatant using RNeasy mini kit (Qiagen) according to the manufacturer's instructions.

All samples were then tested for the presence of WNV RNA by a real-time reverse-transcription PCR suitable for lineage 1 and 2 detection and targeting NS2A (*1*). The assay was carried out with QuantiTect Probe RT-PCR kit (Qiagen) in a total reaction volume of 25 µL with 5 µL of RNA, 0.8 µM and 0.25 µM final concentrations of primers and probe respectively. Cycling conditions were: 50°C for 30 min, 95°C for 15 min followed by 45 cycles at 95°C for 30 sec, 55°C for 30 sec and 72° for 30 sec. USUV RNA was detected by a rRT-PCR targeting NS5 (*2*). The assay was carried out with QuantiTect Probe RT-PCR kit (Qiagen) in a total reaction volume of 25 µL with 5 µL of RNA, 0.8 µM and 0.2 µM final concentrations of primers and probe respectively, and supplemented with a final concentration of 1 mM $MgCl_2$. Cycling conditions were 50°C for 30 min, 95°C for 15 min followed by 45 cycles at 95°C for 15 sec, 60°C for 30 sec. USUV positivity was confirmed by a second rRT-PCR assay targeting NS1 (*3*). The PCR reaction and cycling conditions were similar to the initial USUV screening assay, except for 0.4 µM final concentration of primers and no additional $MgCl_2$.

USUV Genome Sequencing

RNA from the positive brain sample was first reverse transcribed into cDNA using Superscript III Reverse transcription (Invitrogen, Merelbeke, Belgium) and random hexamers (Invitrogen). The complete polyprotein coding sequence of USUV was then sequenced by amplifying overlapping amplicons using previously published (*4*) or newly designed primers (Appendix Table). Purified amplicons were sequenced on an ABI 3130 Avant capillary sequencer (Applied Biosystems) using PCR primers as sequencing primers. Contigs were assembled with Geneious Prime v2019.1.1 (Biomatters, Auckland, New Zealand) by aligning individual sequences to a similar reference strain (GenBank KY294723) identified by blasting amplicon sequences covering NS5. The consensus sequence of the complete polyprotein coding region was then used for phylogenetic analyses (GenBank accession no. OU674388).

Phylogenetic Analyses

All USUV sequences publicly available on 03.08.2021 were downloaded from GenBank into Geneious software. The curated sequence set (after removing sequences not belonging to USUV and clones) contained 843 partial and complete polyprotein coding sequences. All strains were renamed as follows: GenBank accession number_host/country/isolate/year. Given the non-uniform sequencing coverage obtained in various studies, phylogenetic analyses were performed on all complete polyprotein coding sequences (10,305 bp; n = 296) as well as partial sequences to obtain the best representation of the phylogenetic relationship of the strain from Luxembourg. For this purpose, all sequences longer than 500 bp were aligned using MAFFT as implemented in Geneious software. The most commonly sequenced regions were included and comprised of partial NS5 (nucleotides 9088–9597, 510 bp; n = 376) and partial envelope sequences (nucleotides 1003–2067,1065 bp; n = 377). All sequences with unresolved regions (Ns) were removed from the alignments. Identical partial NS5 and envelope sequences were summarized by including only one unique sequence identified thanks to the DNACollapser tool available in FaBox (*5*). The best substitution model fitting each alignment was identified using MEGA v6.06 (*6*). The model with the lowest Bayesian Information Criterion, considered to best describe the substitution pattern observed in the alignment, was then implemented to calculate phylogenetic trees with the Maximum Likelihood method and 500 bootstrap replicates in MEGA v6.06. Preliminary phylogenetic analyses showed that trees based on partial sequences lacked the power to define some lineages and the Africa 3 sublineages with confidence (bootstrap values at main

lineage defining nodes <75% and polyphyletic lineages) and led to some classification discrepancies (data not shown). Therefore further analyses focused on phylogenetic trees calculated on complete polyprotein coding sequences (Appendix Figure).

References

1. Eiden M, Vina-Rodriguez A, Hoffmann B, Ziegler U, Groschup MH. Two new real-time quantitative reverse transcription polymerase chain reaction assays with unique target sites for the specific and sensitive detection of lineages 1 and 2 West Nile virus strains. J Vet Diagn Invest. 2010;22:748–53. PubMed https://doi.org/10.1177/104063871002200515

2. Weissenböck H, Bakonyi T, Rossi G, Mani P, Nowotny N. Usutu virus, Italy, 1996. Emerg Infect Dis. 2013;19:274–7. PubMed https://doi.org/10.3201/eid1902.121191

3. Jöst H, Bialonski A, Maus D, Sambri V, Eiden M, Groschup MH, et al. Isolation of Usutu virus in Germany. Am J Trop Med Hyg. 2011;85:551–3. PubMed https://doi.org/10.4269/ajtmh.2011.11-0248

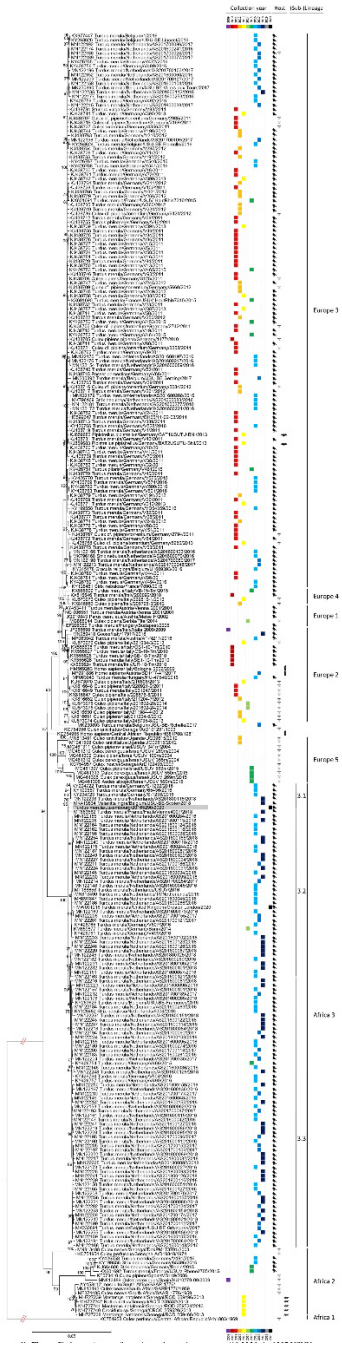
4. Bakonyi T, Gould EA, Kolodziejek J, Weissenböck H, Nowotny N. Complete genome analysis and molecular characterization of Usutu virus that emerged in Austria in 2001: comparison with the South African strain SAAR-1776 and other flaviviruses. Virology. 2004;328:301–10. PubMed

5. Villesen P. FaBox: an online toolbox for fasta sequences. Mol Ecol Notes. 2007;7:965–8. https://doi.org/10.1111/j.1471-8286.2007.01821.x

6. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30:2725–9. PubMed https://doi.org/10.1093/molbev/mst197

Appendix Table. Additional primers designed to sequence USUV complete polyprotein coding region

Primer name	Primer orientation	Primer sequence (5′-3′)
Usu1464F	Forward	CACAACTAGGAGCATCAC
Usu1805R	Reverse	TGCCCGAGAAAGACACTGGA
Usu1726R	Reverse	TGCTTTGTGGCATGGGGCT
Usu3489F	Forward	GGAATGGAGATAAGACCCATGA
Usu3880R	Reverse	CATCTGAAAGAATGCTGCCC
Usu3559F	Forward	AGTGACATGATTGATCC
Usu3854R	Reverse	AGCAGGATGTTCTCTTGG
Usu79F	Forward	AACACAGTGCCGGCAGTTT
Usu162F	Forward	ATGCTGAAACGCGGCATACC
Usu245R	Reverse	GGCCAGCACGAATCGCACT
Usu341F	Forward	CACGGCAATGAAACACCTG
Usu391R	Reverse	CCGATTGTTGACCACGTTGA
Usu467F	Forward	CATGACGGCTGTTTCA
Usu533R	Reverse	CATGTCAGTCGCGTTGATG
Usu673R	Reverse	CAATGTCTTCTGGGTCAT



Appendix Figure. Phylogenetic tree of complete polyprotein coding sequences (10 305 bp) of 297 USUV sequences. The tree was calculated with Maximum Likelihood method and the GTR+G+I substitution model with 500 bootstrap replicates. Only bootstrap values ≥75% are shown. The scale represents the number of substitutions per site. For graphical representation, the long branch to the root of the tree was collapsed. The collection year(s) of the strains are represented with a color code spanning from 2009 to 2020 while hosts (bird, mosquito, human, bat, rodent) are shown with pictograms. The USUV strain from Luxembourg is highlighted with a gray box. Pictograms were designed by Freepik and used under license free terms.